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Rolling ES Cells Down the Waddington Landscape with Oct4 and Sox2

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Embryonic stem cell (ESC) pluripotency is maintained by core transcriptional circuits whereby critical factors sustain their own expression while preventing the expression of genes required for differentiation. Thomson et al. (2011) now show that two core components of the pluripotency circuit, Oct4 and Sox2, are also critical for germ layer fate choice.

In the primordial embryo of most eutherian mammals, the inner cell mass (ICM) hosts embryonic stem cells (ESCs) that can maintain pluripotency in culture in the presence of leukemia inhibitory factor (LIF) and bone morphogenetic protein (BMP) (Smith et al., 1988; Williams et al., 1988). The maintenance of ES cell self-renewal requires a network of transcription factors, including Oct4, Sox2, Nanog, Esrrb, Tbx3, and Klf5. These factors repress genes that promote differentiation and activate genes that maintain pluripotency. Thomson et al. now provide insight into how this pluripotency circuit is deconstructed when LIF and BMP are withdrawn in the presence of specific differentiation signals, allowing ESCs to differentiate into progenitor cells corresponding to different germ cell layers.

Although some factors have been replaced by various combinations of other factors or by small molecules in somatic cell reprogramming using induced pluripotent stem (iPS) technology (Takahashi and Yamanaka, 2006), Oct4 remains the sole factor that, until recently, could not be replaced or omitted. Oct4 belongs to the Octamer class of transcription factors bearing a POU domain and a homeodomain. It acts initially as a maternal factor in the oocyte and remains active in embryos throughout the preimplantation period. The precise levels of Oct4 govern distinct fates of ESCs: a < 2-fold increase in expression causes differentiation into primitive endoderm and mesoderm, whereas repression of Oct4 induces loss of pluripotency and dedifferentiation to trophectoderm. Thus, it appears that a critical amount of Oct4 is required to sustain stem cell self-renewal and that up- or downregulation induces divergent developmental programs, suggesting a role for Oct4 as a master regulator of pluripotency as well as lineage commitment (Niwa et al., 2000). The molecular mechanisms involved in these lineage choices, however, remained unknown.

In order to extend these observations, Thomson et al. established a method whereby withdrawal of LIF and BMP for 48 hr in a carefully controlled medium made the cells competent to respond to differentiation signals (Jackson et al., 2010). By measuring established markers for the mesendodermal (ME) and the neuroectodermal (NE) fates, they showed that the addition of Wnt3a or the Wnt agonist CHIR induced cells to differentiate in the ME fate, whereas the addition of retinoic acid or FGF drove NE differentiation. By integrating genome-wide RNA expression profile data with single-cell measurements, the authors found that, among few other genes, Oct4 mRNA was overexpressed in differentiated ME cells and repressed in NE cells, whereas Sox2, another transcription factor required for pluripotency, showed the opposite behavior. They further showed that Oct4 protein is overexpressed in ME cells and Sox2 in NE cells. This result was confirmed by using Oct4-mCytrine, a fluorescent reporter that allowed them to image live cells undergoing differentiation and track Oct4 levels in real time. As observed for fixed cells, live imaging of ESCs differentiating toward NE fate showed a rapid reduction in Oct4-mCytrine, whereas ESCs differentiating toward ME fate showed a fast induction of Oct4-mCytrine. Importantly, Oct4 modulation occurred way before the earliest known ME or NE lineage-specific markers could be detected, suggesting a role for Oct4 in controlling early steps of ESC transition to ME fate.

Thomson et al. also suggest a role for active Nanog downregulation in differentiation. This idea comes from analysis of the 48 hr lag phase that is required after withdrawal of LIF and BMB before cells start to respond to differentiation signals. During this transition phase, the levels of most of the pluripotency transcription factors were reduced, but the fastest decay was that of Nanog, which fell at a speed quicker than the speed of cell division. It is intriguing to note that, even in this condition, the rate of differentiation into ME or NE was not higher than 70%, suggesting that stochastic cell-to-cell fluctuations in critical factor concentrations might affect the outcome of the process.

Having established that the modulation of Oct4 and Sox2 levels occurs early during differentiation, the authors determined the functional roles of Oct4 and Sox2 in lineage choice at the molecular level. In ESCs, Oct4 and Sox2 were shown to cobind specific targets, imposing either transcriptional repression or activation (Ang et al., 2011; Kim et al., 2008). Surprisingly, however, in differentiated ESCs, each of the two factors was bound to specific target genes. Oct4 bound to a region of the *Sox2* promoter in the ME lineage, whereas Sox2 bound to the promoter region of *Brachyury* in the NE lineage, suggesting that each of the two factors represses key genes of

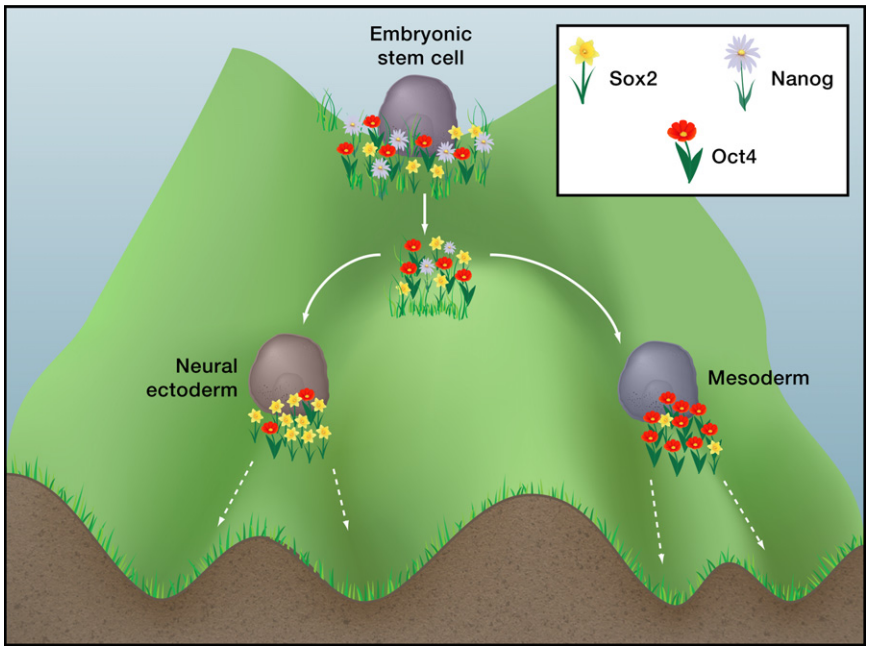

Figure 1. Progressive ESC Differentiation into Germ Layer Progenitors by Breakdown of the Pluripotency Transcriptional Circuit

In the concept of epigenetic landscape of Conrad H. Waddington, differentiating cells during development are assimilated to marbles rolling down the valleys toward the points of lowest elevation. During development, cells make choices that depend on their history as well as on developmental and environmental inputs. High hills represent energy barriers that prevent differentiated cells from suddenly changing their fates. Likewise, upon induction of differentiation, ESC progenies (represented at the top of the hill) unfold their developmental program by selecting progenitor fates (bottom cells), that further progress toward terminal differentiation. ESC fate requires a pluripotency circuit that is represented as a flower landscape containing a defined amount of Oct4, Sox2, and Nanog. As cells roll down the hill, they reach a first intermediate state with low Nanog that makes them responsive to differentiation signals. These signals induce an asymmetry, with higher Oct4 driving the ME progenitor cell fate, whereas higher Sox2 induces the NE progenitor cell fate.

the unwanted differentiation state. Moreover, Oct4 was shown to bind a region of the *Brachyury* promoter that is activated in the ME lineage. Thus, both Oct4 and Sox2 are redeployed by differentiation signals to induce a differentiation pathway while repressing the others. That this redeployment is critical is emphasized by the fact that forced overexpression of Oct4 in cells induced to NE differentiation prevented them to express the NE marker Sox1 (Abranches et al., 2009), whereas forced Sox2 expression in cells induced to the ME fate prevented them to express the ME marker Brachyury (Yamaguchi et al., 1999), showing that differentiation into one fate requires downregulation of the crucial factor determining the other one.

These results can be interpreted in analogy to the scenario of epigenetic landscape first proposed by Conrad H. Waddington, in which pluripotent cells would resemble marbles rolling down a hill, making choices at key branching points in order to reach their differentiated state (Figure 1). When Nanog is present together with Oct4 and Sox2, these factors sustain ESC pluripotency, but when Oct 4 or Sox2 are alone, they can play a causal role in the determination of the ME and the NE pathways, respectively. The branching points are thus represented by concentration changes in critical differentiation factors induced by developmentally induced signals. While clarifying how ESCs are sent toward the NE or ME fate, this work also raises many questions. How do the differentiation signals induce specific repression of one of the two proteins without repressing the other one? How is targeting of Oct4 and Sox2 differentially regulated in ESCs versus differentiated cells, and how is each of these two factors simultaneously inducing repression at certain genes while activating others? The identification of the Oct4 and Sox2 partners in each cell type may help us to understand these questions. Moreover, the generation of genome-wide ChIP-seq profiles for Oct4 and Sox2 and active versus repressed chromatin marks in the ME and NE cells may help to decipher how these factors can determine each of the two progenitor states. Also, it takes 48 hr and several cell divisions for ESCs to become responsive to differentiation signals. What is happening in the cell during this time and how it is important for differentiation are important questions. A key event seems to be the rapid, active downregulation of Nanog. Future studies should aim to unravel the molecular mechanisms that are responsible for this rapid decay, as well as establish whether other processes concur concomitant with Nanog downregulation to prime cells for differentiation. Because the authors detected expression changes in chromatin regulators, such as Jarid2 and DNA methyltransferases, their potential roles in the transition phase and during differentiation should be better elucidated. For all of these questions, the availability of carefully controlled systems where isogenic cells can efficiently take one or the other differentiation route in a timely coordinated fashion will be of crucial importance. A better understanding of how pluripotent cells roll down the Waddington landscape upon appropriate stimulation may also lead, in the longer term, to the definition of more efficient conditions for the reverse task of reprogramming differentiated cells into pluripotent conditions.

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A Kinesin in Command of Primary Ciliogenesis

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Primary cilia sense extracellular cues and in response transmit signals required for development and tissue homeostasis. A new study by Kobayashi et al. (2011) reports that the kinesin Kif24 controls the formation of primary cilia by restricting the nucleation of cilia at centrioles.

Centriole duplication and function are temporally coupled with the cell cycle. In cycling cells, centrioles function as the centrosome core for cytoplasmic microtubule organization, whereas in quiescent cells (G_0) and cells in G_1, the centriole functions as a basal body for the nucleation of axoneme microtubules of the primary cilium. This switch in function is important for early development (the basal bodies of the sperm flagellum become centrioles after fertilization) and for the proper coordination of chemical and mechanical signaling events through different stages of both development and the cell cycle.

New studies have begun to uncover the mechanisms that control the centriole to basal body switch. The cyclin-dependent kinase substrate CP110 is a previously known regulator of primary ciliogenesis that was initially identified for its role in centrosome duplication (Spektor et al., 2007). In complex with Cep97, CP110 localizes to the distal end of centrioles during the assembly of new centrioles. During ciliogenesis, CP110 is specifically eliminated from the mother centriole—the one destined to become the basal body for the primary cilium. CP110-Cep97 levels are low during G_0, when cilia are formed and depletion of CP110 leads to the promiscuous formation of primary cilia in cycling cells. In order to restrict cilia formation in cycling cells, this complex may cap the distal end of centrioles to limit axoneme microtubule assembly and/or inhibit proteins required for ciliogenesis, such as Cep290 and Rab8a (Kleylein-Sohn et al., 2007; Schmidt et al., 2009; Tsang et al., 2008). However, an enzymatic activity for the CP110-dependent regulation of ciliary axoneme microtubules has not been identified.

The exciting new study by Dynlacht and colleagues (Kobayashi et al., 2011) demonstrates that a member of the kinesin-13 family functions with CP110 to suppress cilia formation. In contrast to canonical kinesin-driven cargo transport along microtubules, kinesin-13 subfamily members regulate microtubule length by disassembling microtubule ends. Humans express four kinesin-13 motor proteins, and the three extensively studied subfamily members (Kif2A, Kif2B, and Kif2C/MCAK) function in mitotic chromosome segregation and neurodevelopment. The fourth deeply conserved kinesin-13, Kif24, has distinct functions. In protists, Kif24 subfamily members localize to flagella, and overexpression or knockdown causes decreased or increased flagella length, respectively (Blaineau et al., 2007; Dawson et al., 2007; Piao et al., 2009). Thus, kinesin-13s also modulate the balance of axoneme microtubule dynamics to maintain normal flagella and ciliary length.

Consistent with this role for Kif24 proteins in protists, the authors show that human Kif24 antagonizes primary cilia formation by regulating centriole microtubules. Kif24 preferentially localizes to the distal end of mother centrioles and coimmunoprecipitates with CP110 and Cep97 (Kobayashi et al., 2011). Kif24 recruits and/or maintains CP110 at mother centrioles, and overexpression of Kif24 restricts ciliogenesis in quiescent cells. In vitro, Kif24 binds to and destabilizes microtubules, though not to the extent